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Prime Editing- A Versatile Genome Editing Technology

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SUMMARY

Prime editing is the most recent gene-editing method, has advantages above traditional CRISPR. In traditional CRISPR, Cas9 breaks both strands of the DNA, which can also occur in nature, can be detrimental to the cell and need to be quickly repaired. In prime editing, the targeted insertions, deletions, and base-to-base conversions are mediated without the need for double strand breaks (DSBs) in the target region. When compared to the traditional CRISPR-CAS method, it reduces off-target edits and addresses frame shifts brought on by indels.

INTRODUCTION

Prime Editing is a novel and versatile Genome Editing on CRISPR systems that expands the guide RNA's responsibility to guide Cas9 to a targeted genomic location, and acting as an RNA template for incorporating new sequences into the DNA genome (Anzalone, 2019). In addition to maintaining the elegantly simple two-molecule CRISPR approach (Cas9 + gRNA), Prime Editing employs an editing mechanism that operates independently of the host cell's homology-directed repair (HDR) machinery is typically utilized to copy information from donor DNA templates. Prime Editing with CRISPR has introduced an unnatural complex, Cas9 fused with a reverse transcriptase, which makes target-primed reverse transcription (TPRT) viable in plant system for small edits (1 to about 40 bases). PE is a ground-breaking technology that can produce accurate transition and transversion mutations and tailored insertions or deletions at targeted genomic regions.

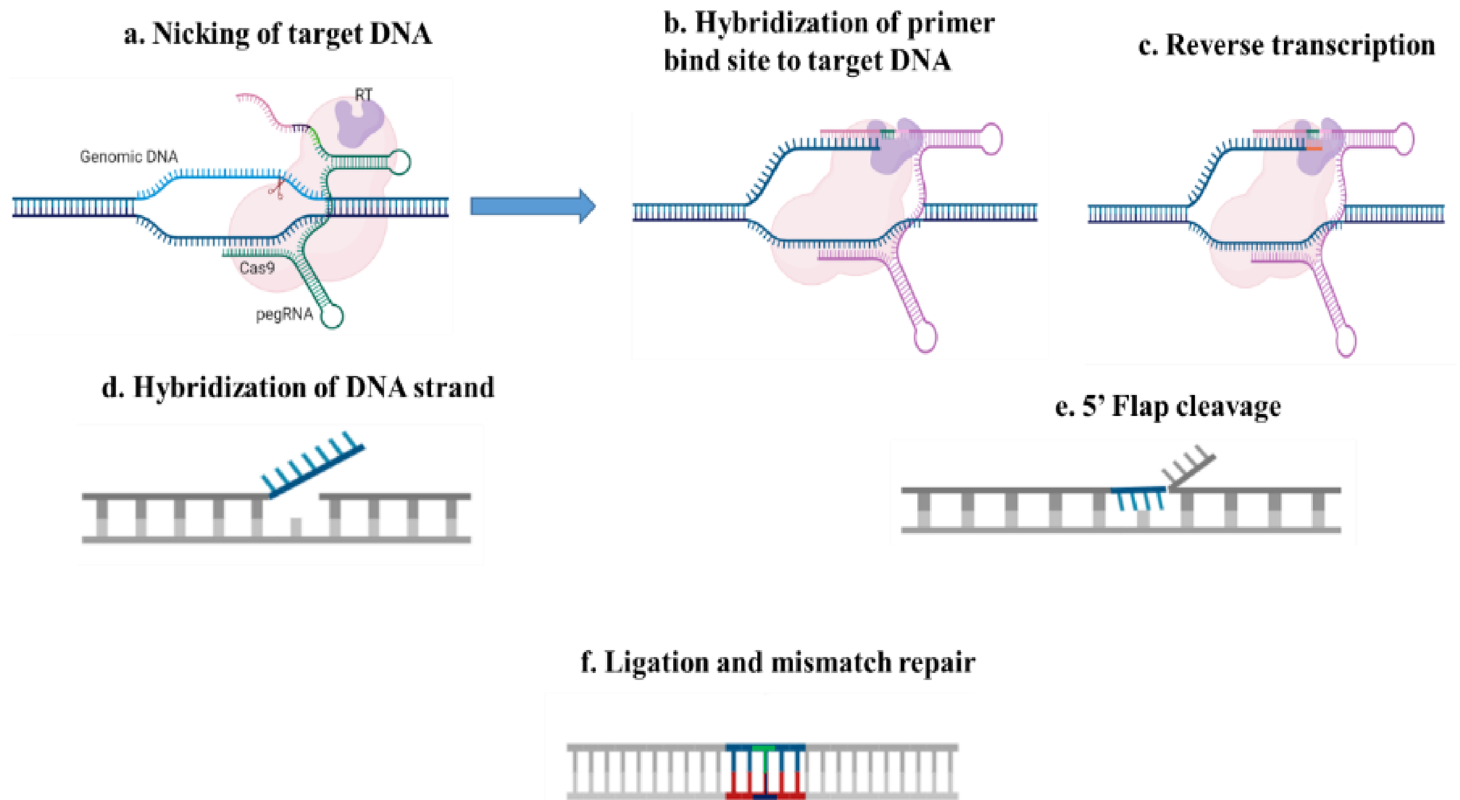
Prime editing strategy:

In the CRISPR Cas9 system, In the CRISPR Cas9 system, the Cas9 protein targets DNA using a guide RNA that contains a spacer sequence that hybridizes to the target DNA region. A prime editing complex consists of a prime editing protein containing an RNA-guided DNA-nicking domain, such as Cas9 nickase, fused to a Reverse Transcriptase (RT) domain and complexed with a pegRNA that encodes the primer binding site (PBS). The PE-pegRNA complex enables a variety of precise DNA edits at a wide range of positions. The PE-pegRNA complex binds the target DNA and nicks the PAM-containing strand. These initial steps result in a branched intermediate with two redundant single-stranded DNA flaps: a 5′ flap containing the unedited DNA sequence and a 3′ flap containing the edited sequence copied from the pegRNA. Although hybridization of the perfectly complementary 5′ flap to the unedited strand is likely to be thermodynamically favored, 5′ flaps are the preferred substrate for structure-specific endonucleases such as FEN1 (Liu et al., 2004), which excises 5′ flaps generated during lagging-strand DNA synthesis and long-patch base excision repair. The redundant unedited DNA may also be removed by 5′ exonucleases such as EXO1 (Keijzers et al., 2015). The resulting 3′ end hybridizes to the primer binding site, then primes reverse transcription of new DNA containing the desired edit using the RT template of the pegRNA and create heteroduplex DNA containing one edited strand and one unedited strand. Equilibrium between the edited 3′ flap and the unedited 5′ flap was done by a DNA repair mechanism to resolve the heteroduplex by copying the information in the edited strand to the complementary strand, resulting in stably edited DNA (Figure 1.)

Schematic Illustration of Prime Editing:

a) The prime editor (PE) composed of a Cas9 fused to a reverse transcriptase (RT) and pegRNA bind to target DNA,
b) The nuclease domain of the editor nicks one DNA strand and binds to the primer binding site on the extended 3′ end of the pegRNA.
c) The RT elongates the nicked DNA strand (incorporating the edit).
d) The replacement of the original sequence via endogenous DNA mismatch repair mechanism incorporates the desired mutation at the target site,
e) A desired edit is installed after DNA repair of the heteroduplex DNA.

Figure 1. Schematic Illustration of Prime Editing



CONCLUSION

Prime editing is a significant advancement in the toolbox of genome editing technologies. It is amost recent technique created to alleviate CRISPR/Cas constraints and calibrate the genome editing process. Prime editing, though, introduces new challenges. Large DNA insertions or deletions may not be possible with prime editors compared to traditional CRISPR/Cas9 systems.In conclusion, prime editing, which offers more accurate base editing ability and efficiency, is undoubtedly another double weapon available in the area of genome editing.

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